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# Challenges in development of an anti-idiotypic cancer vaccine

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# CHALLENGES IN DEVELOPMENT AN anti-IDIOTYPIC CANCER VACCINE

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Early Stage Product Development

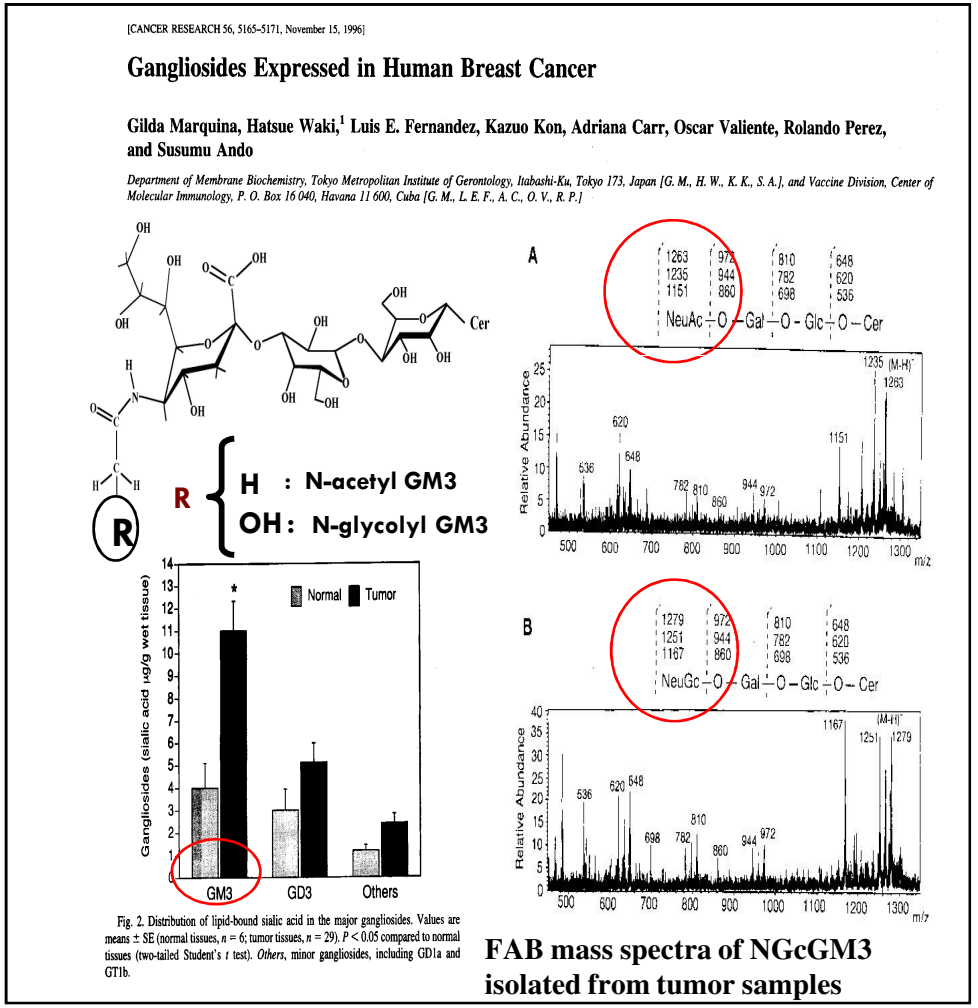
| VACCINE TECHNOLOGY IV
Albufeira, Portugal, May 2012

CIM Centro de Inmunología Molecular

# GANGLIOSIDE TARGETED IMMUNOTHERAPY

- ✓ *A scientific discovery in 1996:* Ganglioside-pattern expressed in human breast cancer *(Marquina et al., Cancer Res 1996, 56(22):5165-5171)*
- ✓ *N*-glycolylneuraminic acid-containing gangliosides are attractive targets for cancer immunotherapy because these glycolipids are non-self antigens in humans *(Irie et al., J Biol Chem 1998, 273(25):15866-15871)*
- ✓ Recent experimental data suggest that N-glycolyl-GM3 ganglioside is relevant for tumor biology *(de Leon et al., Cancer Immunol Immunother 2006, 55(4):443-450)*

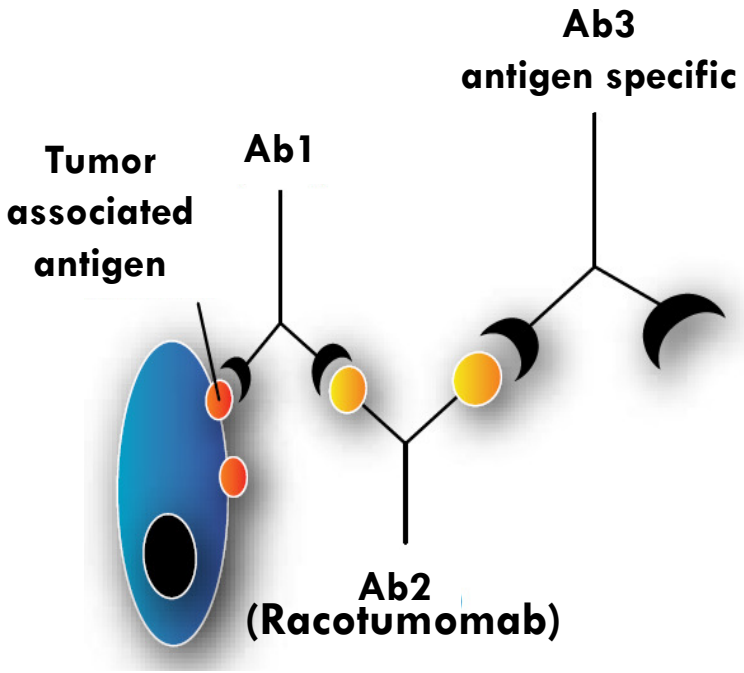
***An emerging concept:* N-glycolylated gangliosides are Tumor Specific Antigens and Idiotypic Network Antigens**




**FAB mass spectra of NGcGM3 isolated from tumor samples**

# TWO NOVEL THERAPEUTIC APPROACHES

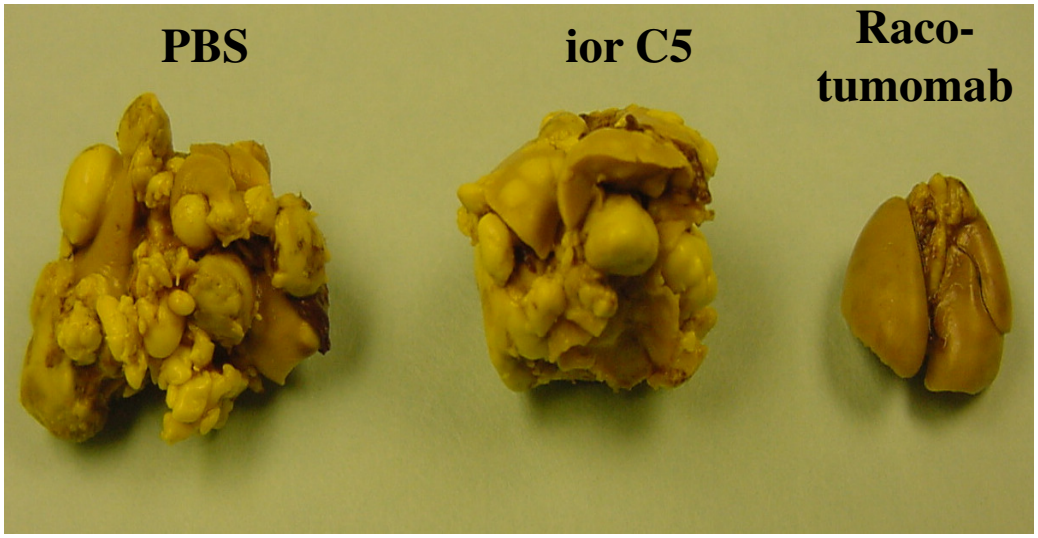
- Hydrophobically incorporated gangliosides into bacteria-derived proteosomes
- Anti-idiotypic monoclonal antibodies as antigen surrogates




- ✓ Racotumomab is an IgG1 anti-idiotype (Ab2) mAb obtained by immunizing Balb/c mice with Ab1 coupled to keyhole limpet hemocyanin (KLH) in the presence of Freund's adjuvant
- ✓ Racotumomab (Ab2) behaves as an antigen internal image of antigen: immune networks discriminates self versus non-self

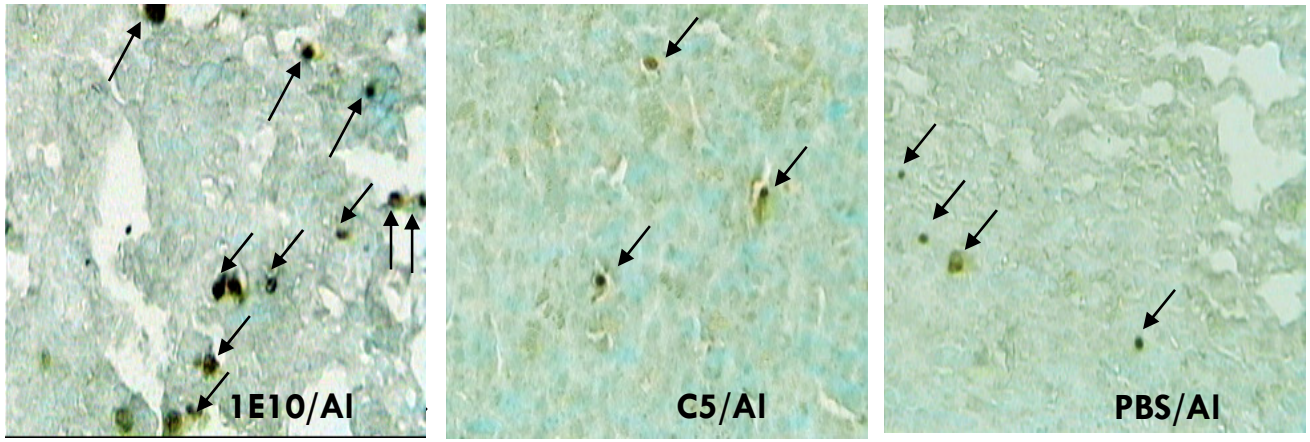
*Vazquez et al., Hybridoma 1995, 14(6):551-556*
*Vazquez et al., Hybridoma 1998, 17(6):527-534.*

# ANTIMETASTATIC EFFECT AND APOPTOSIS INDUCTION BY RACOTUMOMAB IV TREATMENT IN 3LL-D122 TUMOR MODEL




*Alfonso et al., Cancer Biology & Therapy, 2007*
*Fuentes et al., Breast Cancer Res Treat 2009*

*Apoptosis was measured by the Apoptag system methods*

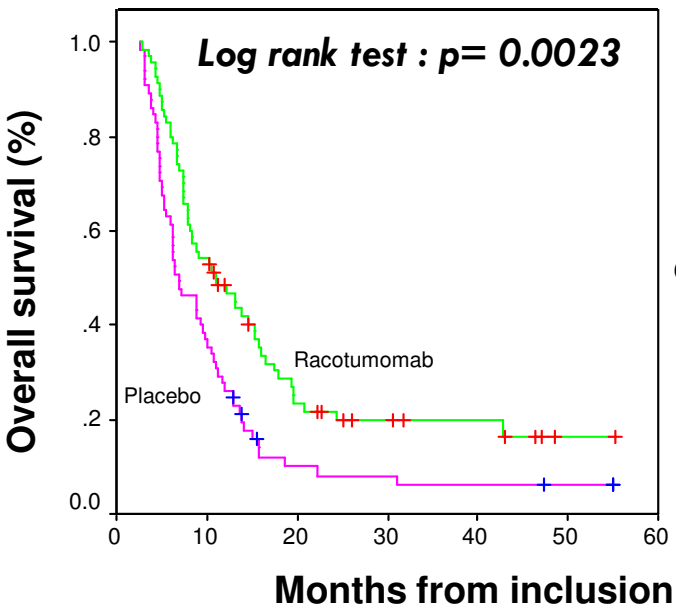



*Diaz et al., Cancer Immunol Immunother 2009, 58(7):1117-1128*

# PHASE II/III CLINICAL TRIAL IN NSCLC

## Multicentric, randomized, double blind and placebo- controlled

**OS Analysis**




*Kaplan-Meier survival curves in relation to treatment. Per Protocol Analysis, ≥5 doses (induction phase)*

*135/174 patients (77,5%)*

*Only patients who completed the induction period of the study (≥ 5 doses) were included in this analysis*

| OS (PPP) | |
|---|---|
| **Group** | **Median, months** |
| **Vaccine (n=70)** | **10.90 (95% CI 6.82 -14.98 )** |
| **Placebo (n= 65)** | **6.90 (95% CI 4.43 –9.37 )** |

*Other Raco Clin. Trials reports: Alfonso et al., J Immunol 2002, 168(5):2523-2529; Diaz et al., Clin Immunol 2003, 107(2): 80-89; Neninger et al., Cancer Biol Ther 2007, 6(2):145-150; Hernandez et al., J Immunol 2008, 181(9):6625-6634.*

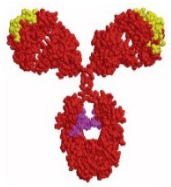
# A MONOCLONAL ANTIBODY AS ANTIGEN: FROM ASCITIS TO BIOREACTOR

Murine Ascitis Fluid

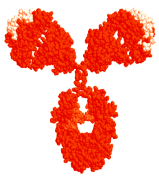
Stirred Tank
15 L, protein free medium
Raco supernatant

Ionic exchange chromatography
Protein A chromatography

Protein A chromatography
Ionic exchange chromatography

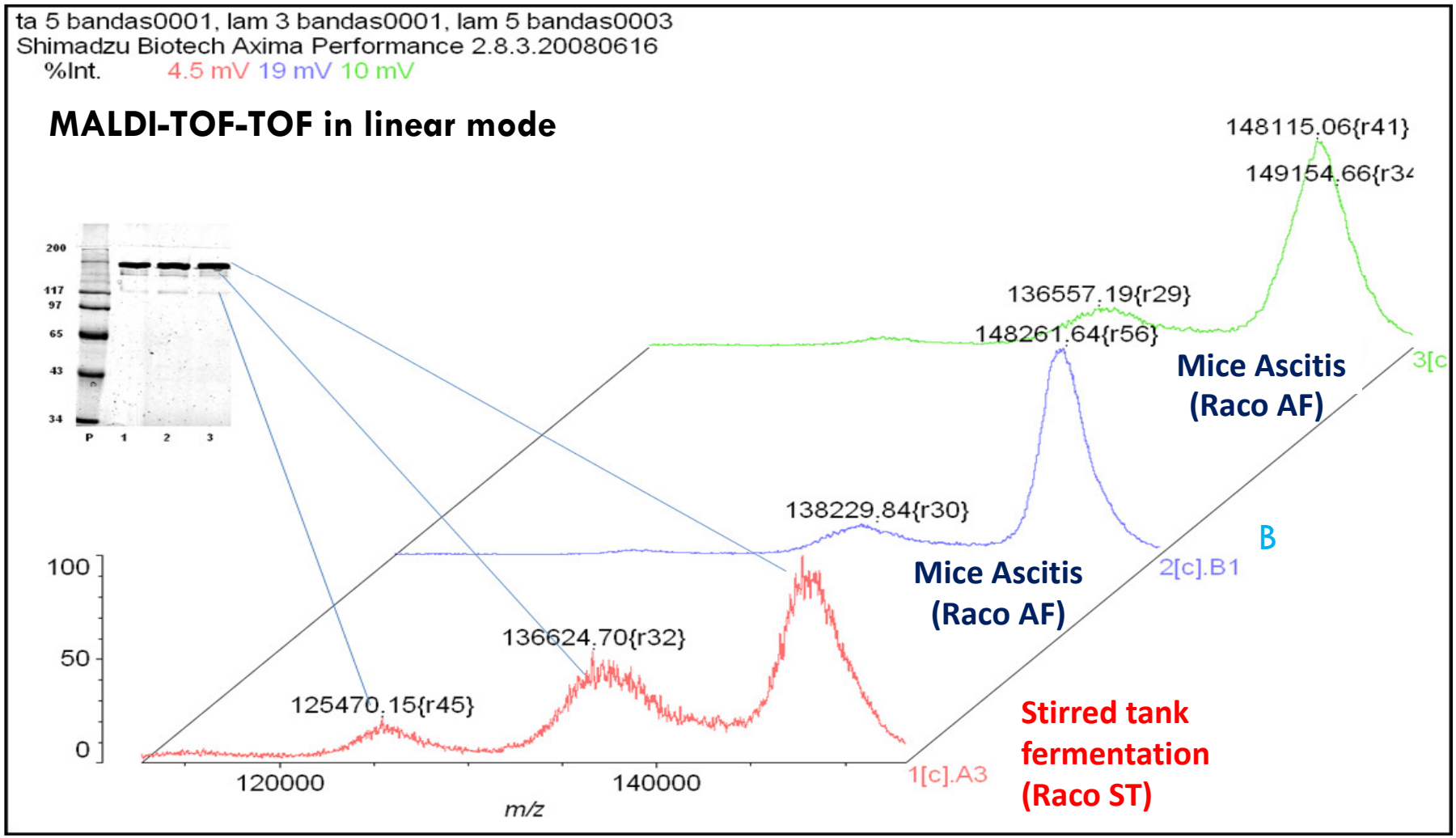


Raco AF

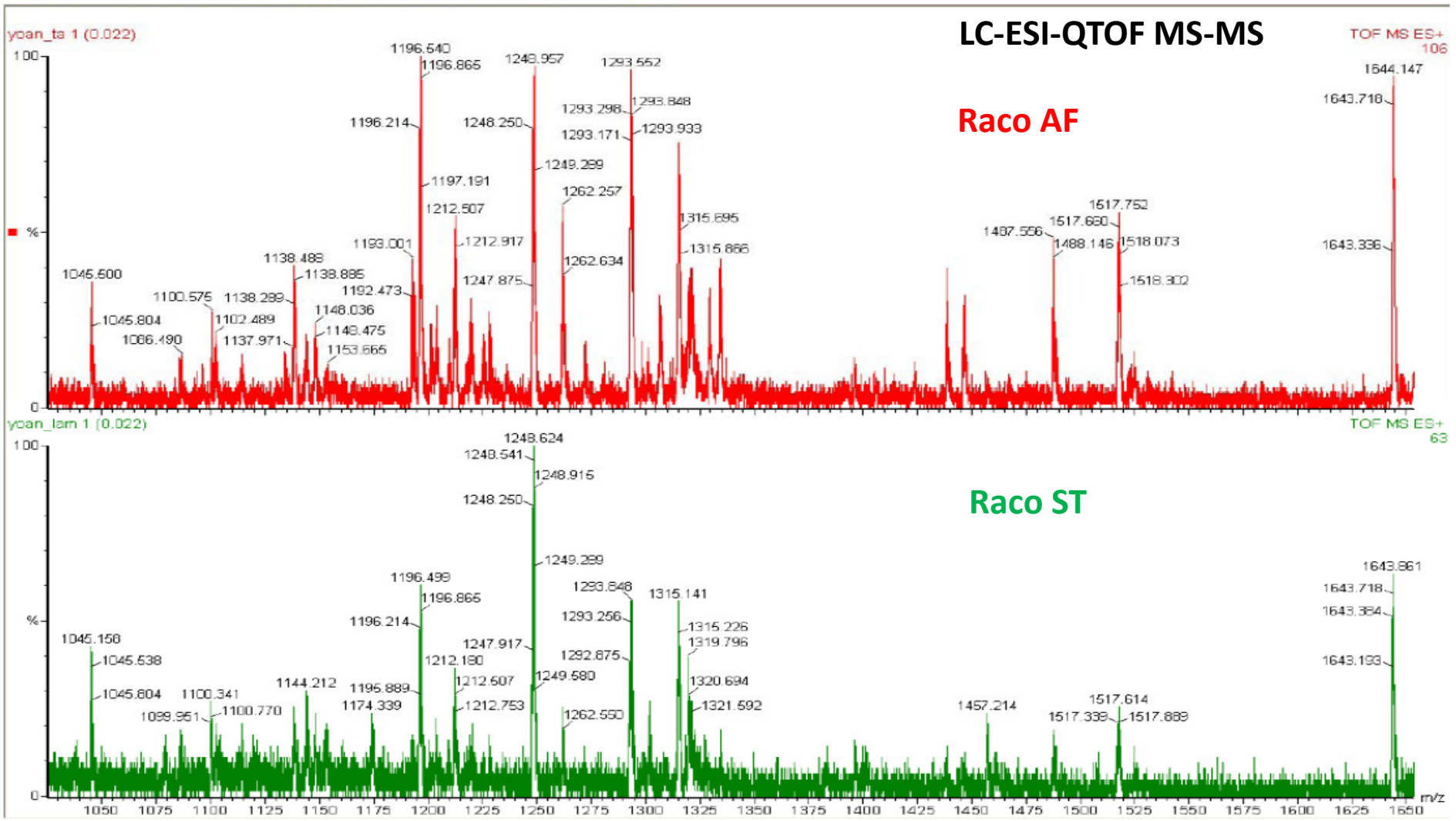


Raco ST

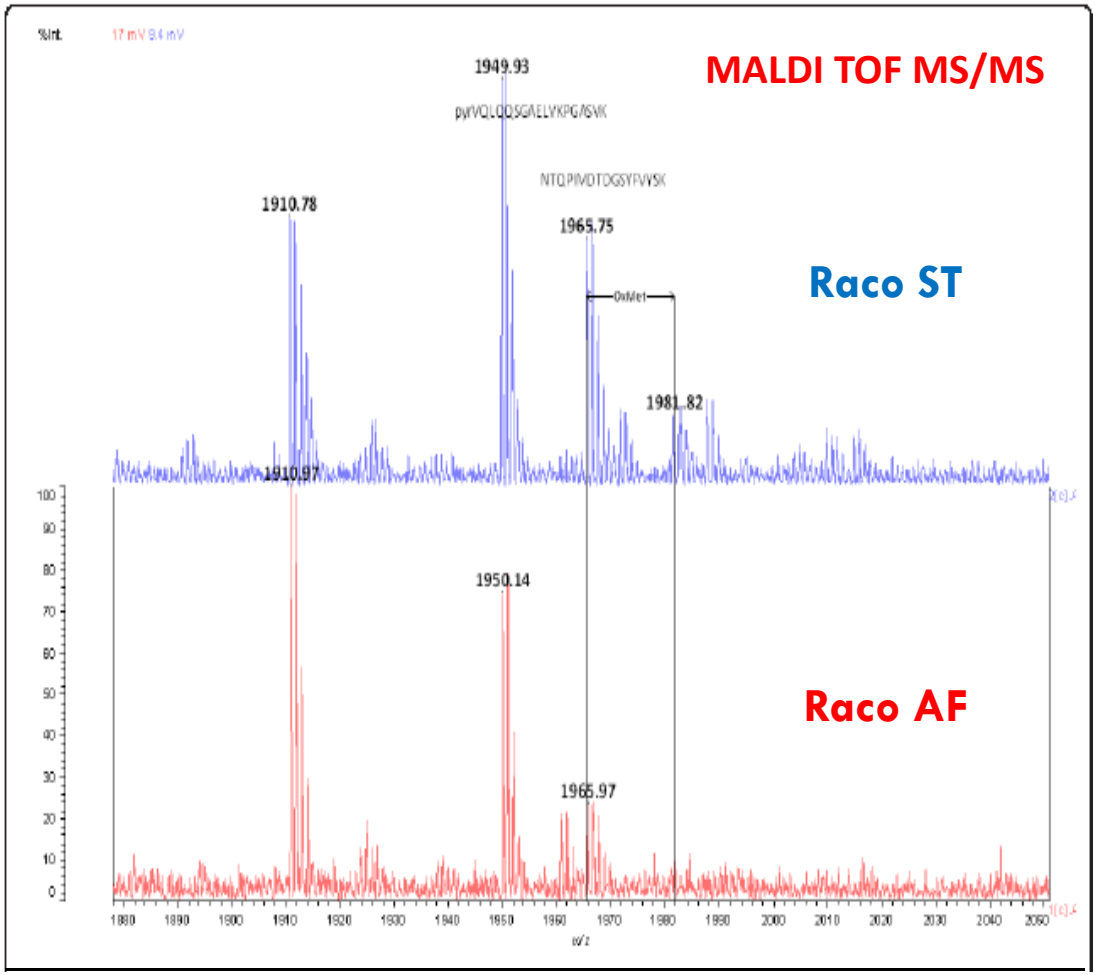
# Both products have the same molecular mass for whole antibody molecule obtained by SDS-PAGE



# Both molecules have the same “peptide mass fingerprinting” pattern indicating high similarity



# The amino acid sequence, N-terminal pyroglutamic acid, Asn glycosylation and three deamidation sites are common for both conditions, while oxidized methionine found only in Raco-ST

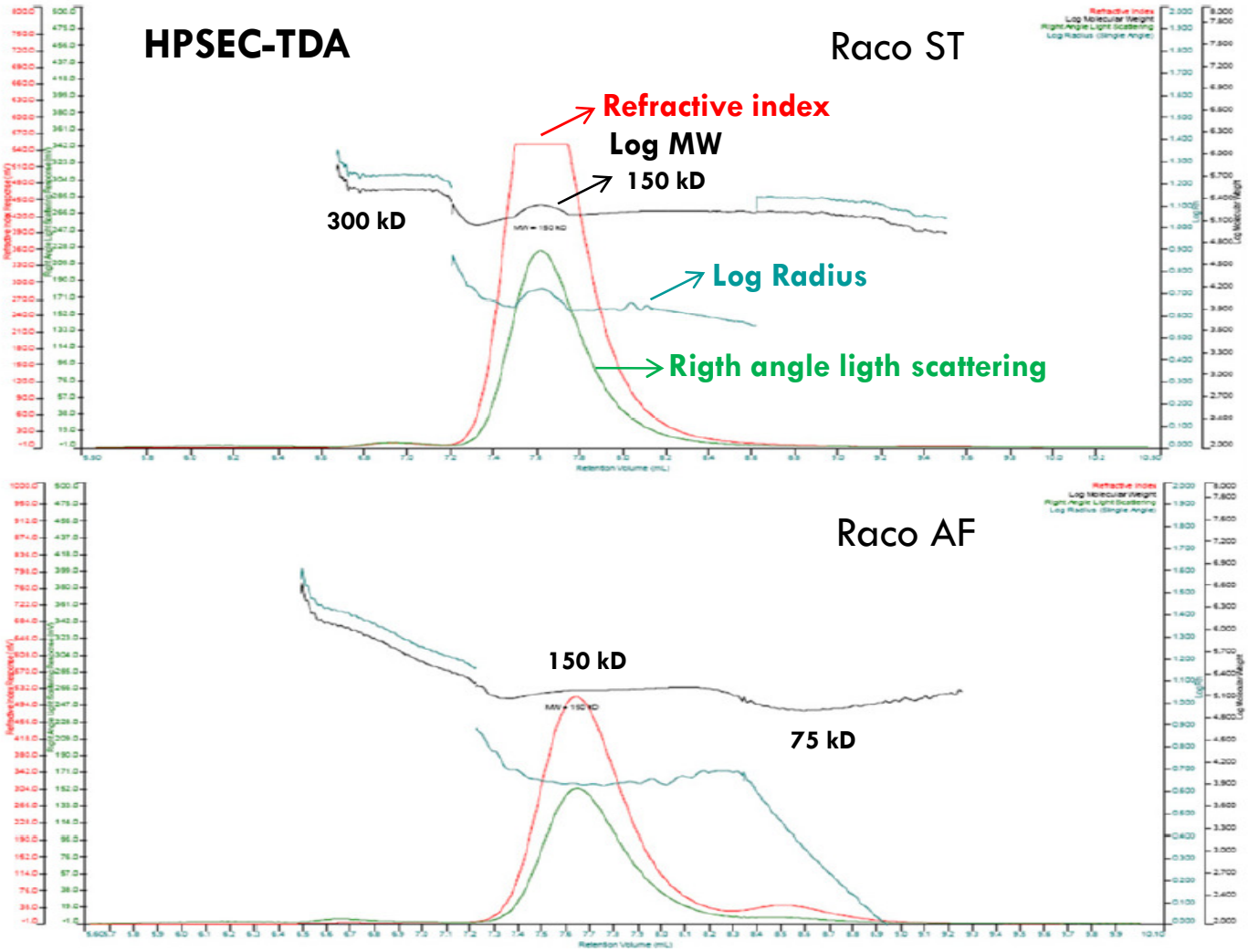



| Modifications | Raco AF | Raco ST |
|---|---|---|
| N-term Gln to pyroglutamic acid | + | + |
| Glycosilation of $Asn_{294}$ | + | + |
| Deamidation of $Asn_{141}$ ($C_H$) | + | + |
| Deamidation of $Asn_{157}$ (CL) | + | + |
| Deamidation of $Asn_{161}$ ($C_L$) | + | + |
| Oxidation of $Met_{396}$ | - | + |

**+ Modified; - Non modified**

*Machado et al. BMC Biotechnology 2011, 11:112*

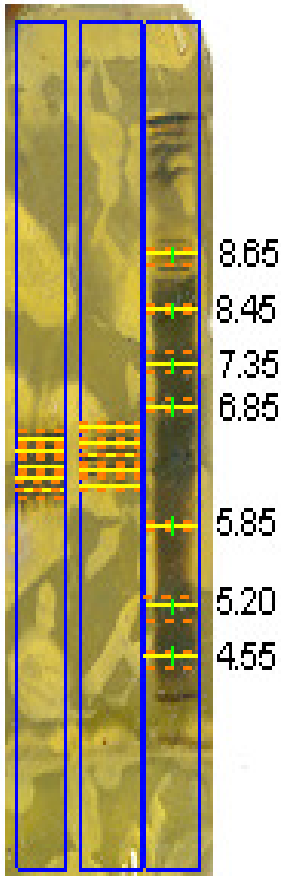
# Very low amounts of aggregates in Raco-AF and ST, but low molecular weight fragments present only in Raco-AF

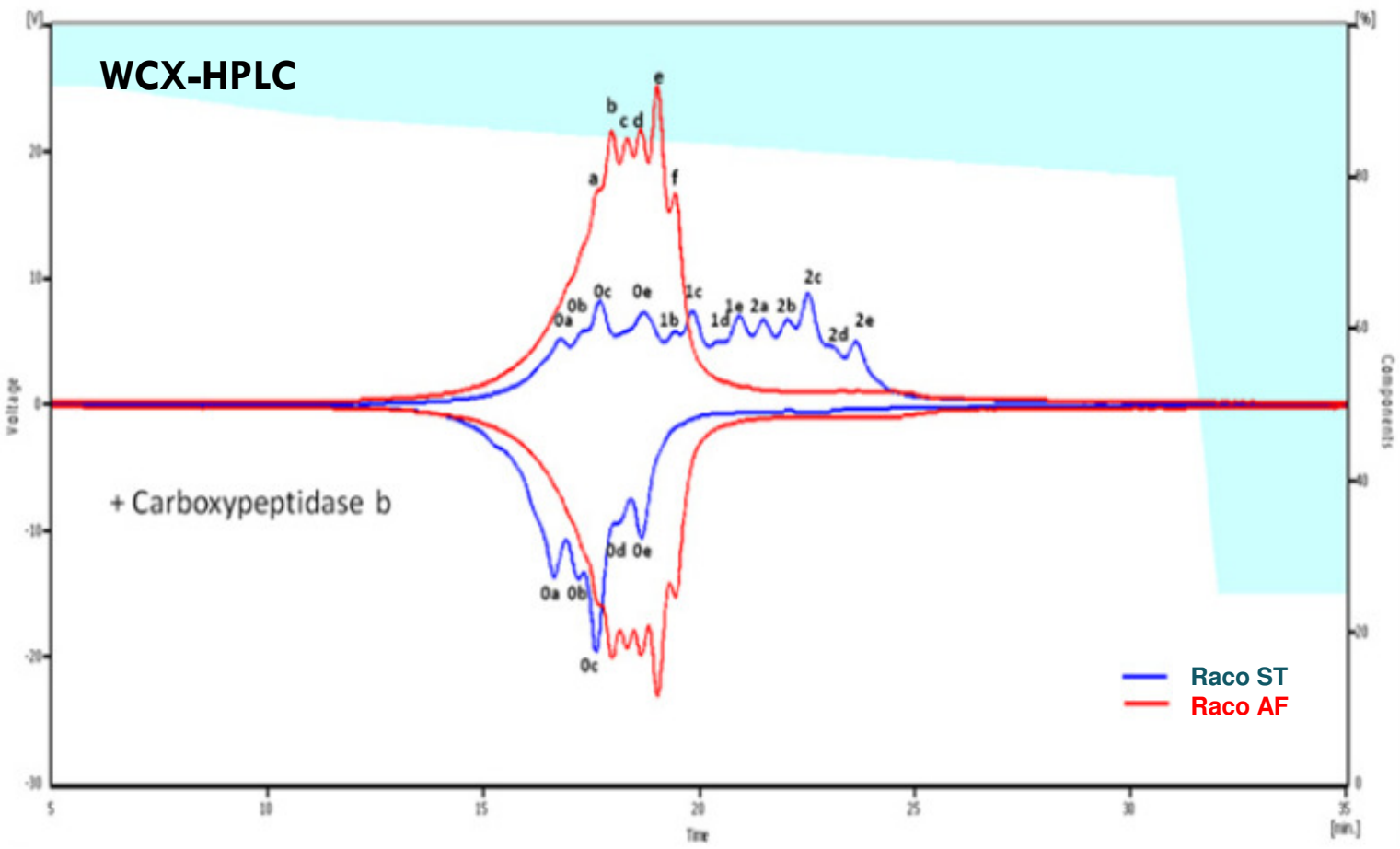



*HPSEC (TSK G2000 WXL in HPI100) UV detector at 280 nm, sequential refractive index (RI), intrinsic viscosity (IV), and right-angle light scattering detection (TDA 302, Viscotek Corp.)*

*Machado et al. BMC Biotechnology 2011, 11:112*

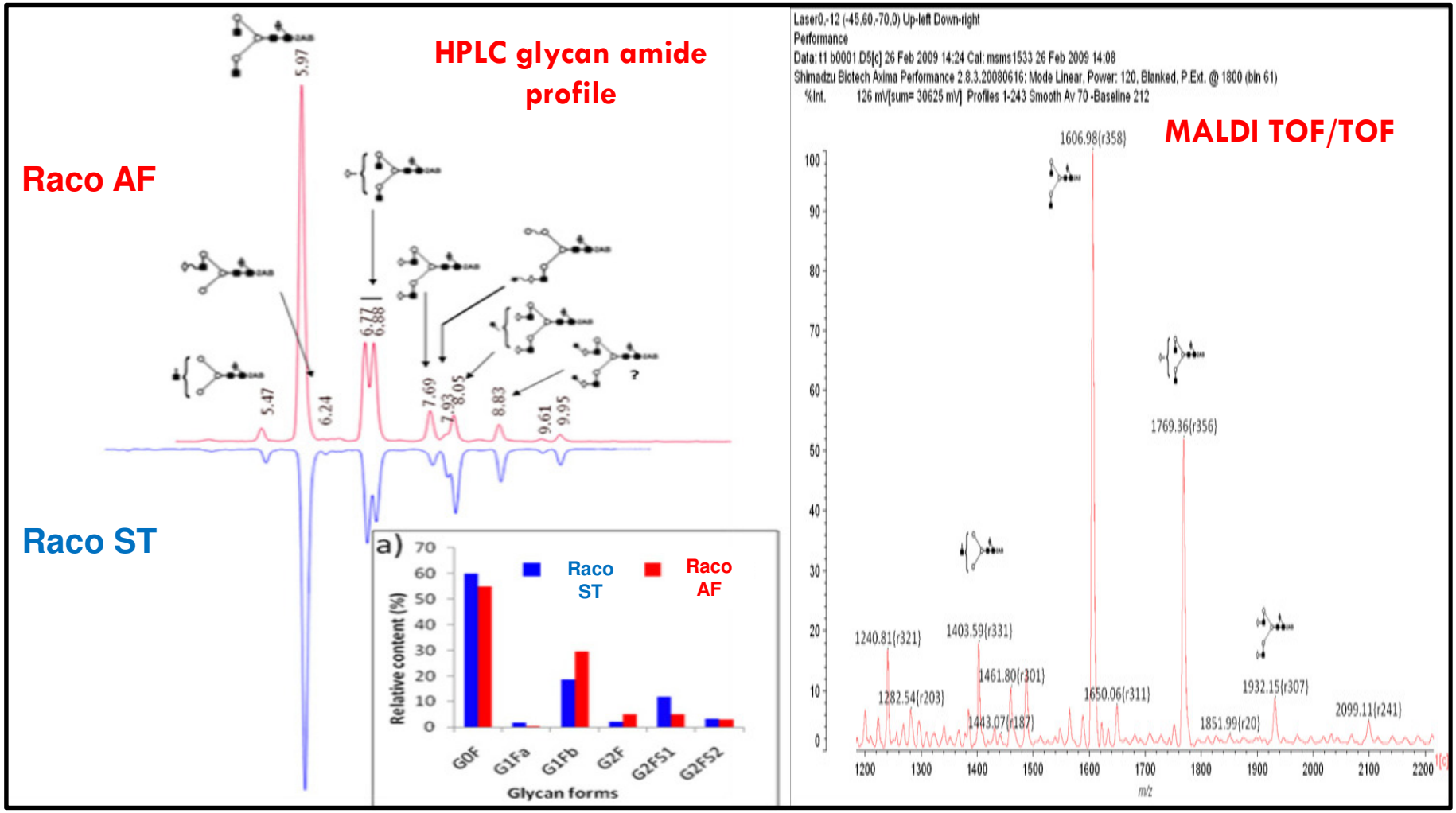
# Slightly more acidic isoelectric point of Raco-ST and different charge profile compared with Raco-AF, even after C-terminal lysine removal



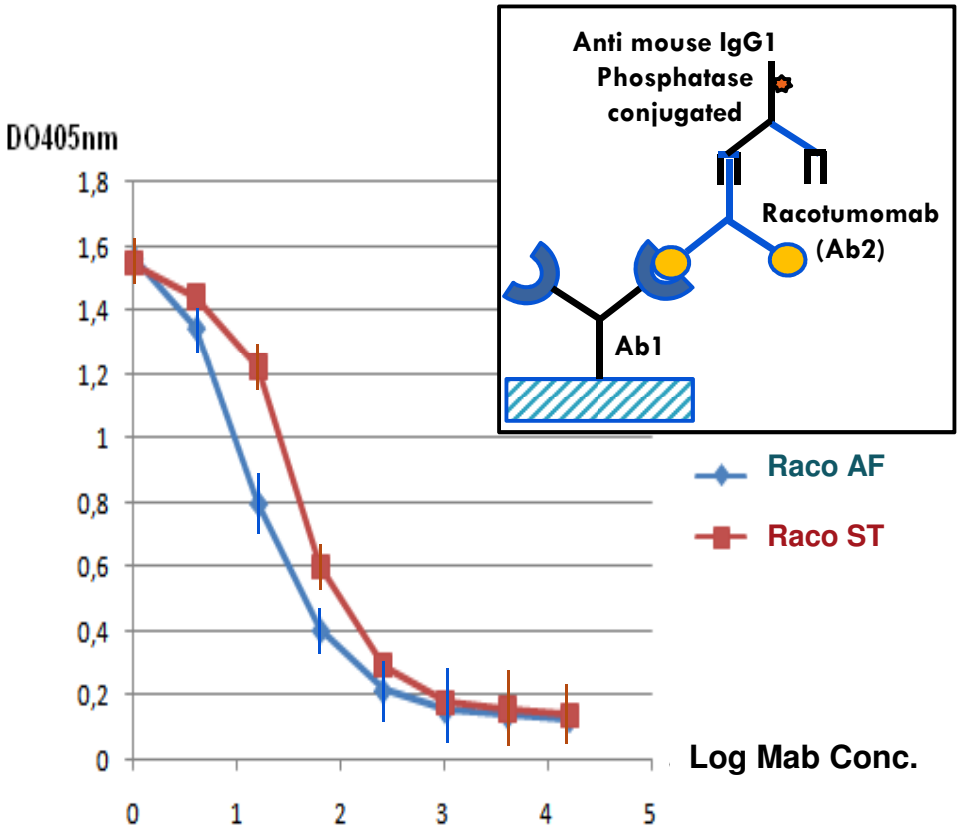

*Machado et al. BMC Biotechnology 2011, 11:112*

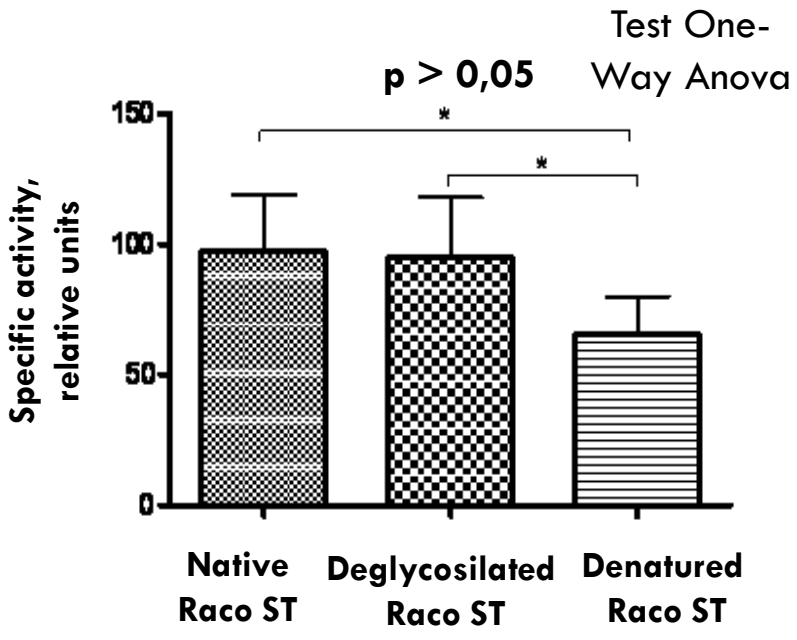
# Same sugar structures attached to Fc of Raco-AF and ST (mainly fucosilated, not bisected in N-acetilglucosamine, mostly G0 and G1 estructures); but not their relative amount




*Machado et al. BMC Biotechnology 2011, 11:112*

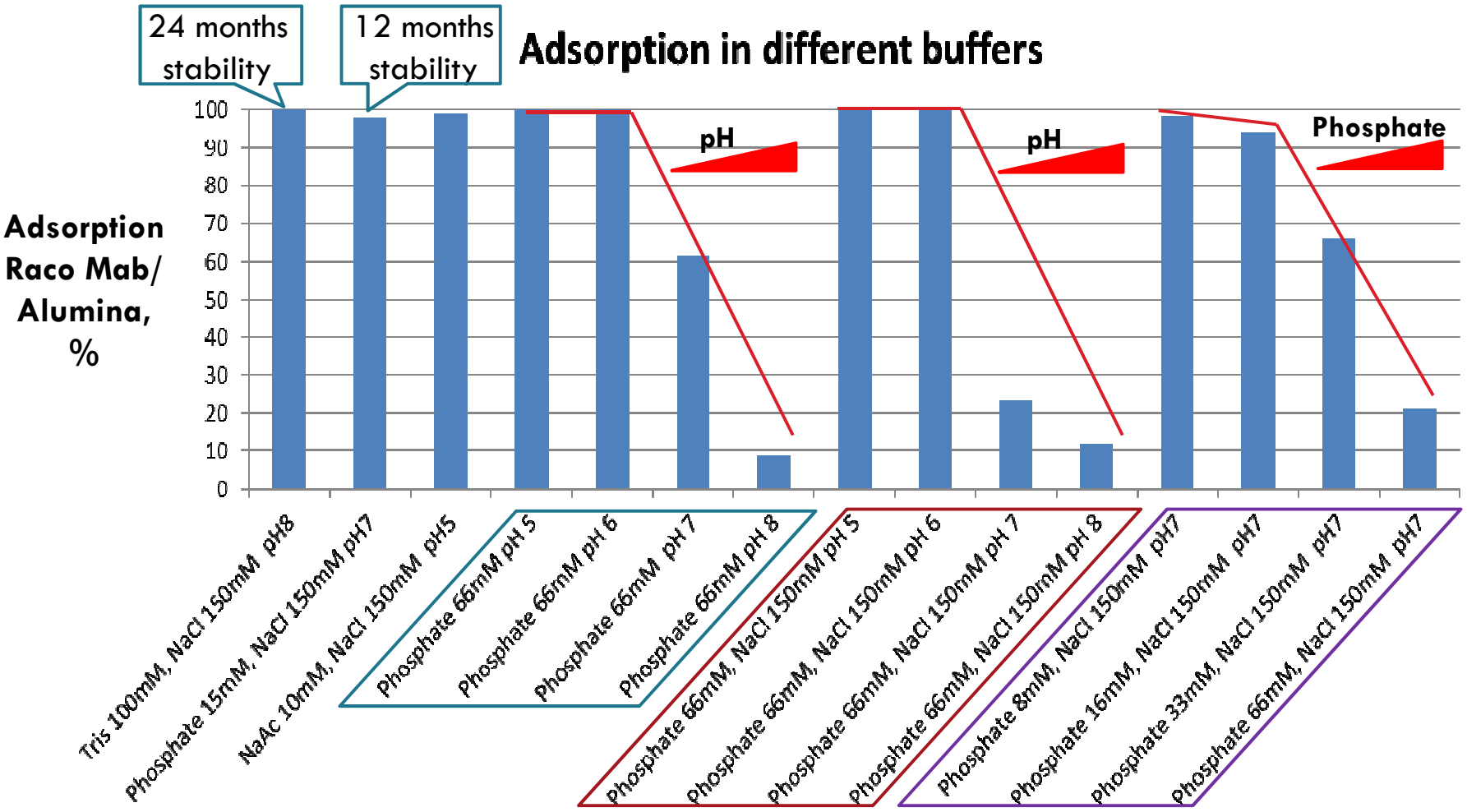
# Raco FA and ST have similar Ab1 recognition profile and deglycosilation with PNGase F didn't affect specific activity



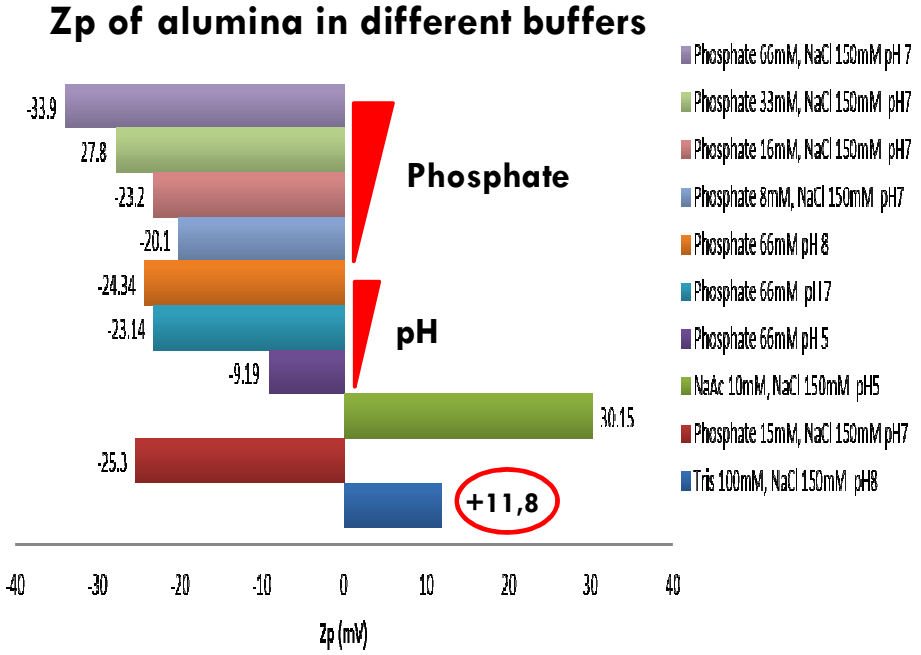



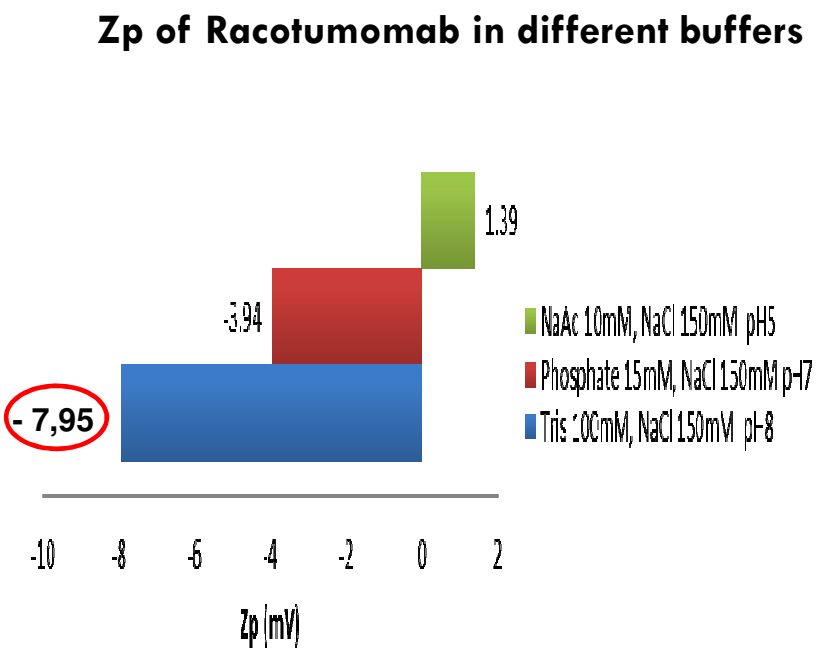

$$\text{Specific activity} = \frac{\text{DO Ab1 recognition}}{\text{DO isotype (IgG1) recognition}}$$

# The presence of phosphate ion in vaccine formulation affects the adsorption for pH values above 7, even when ionic strength is increased



# Increases in phosphate concentration and buffer pH rendered alumina more negatively charged, while Racotumomab became negative when pH increases above 6

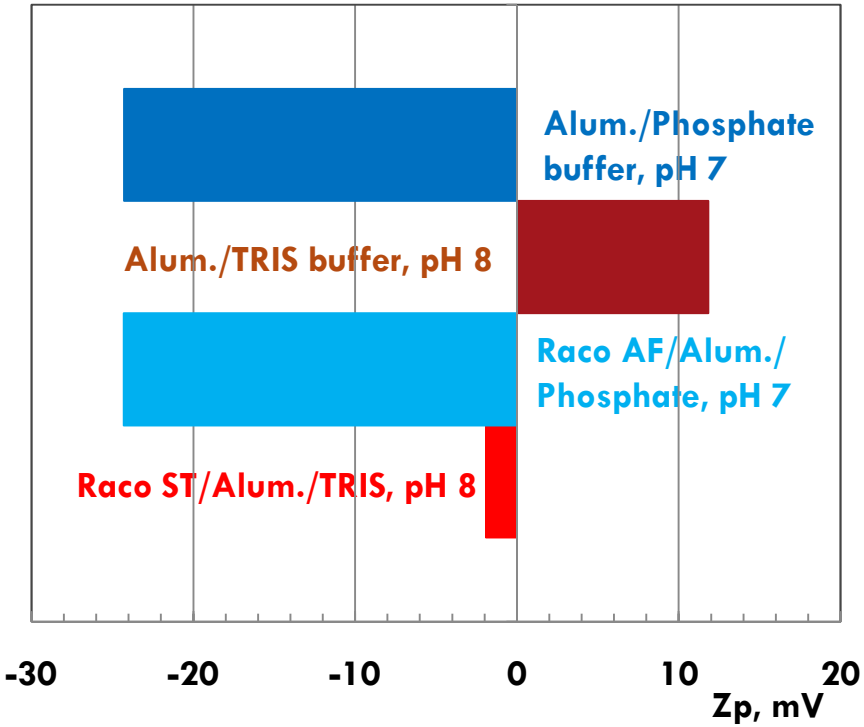


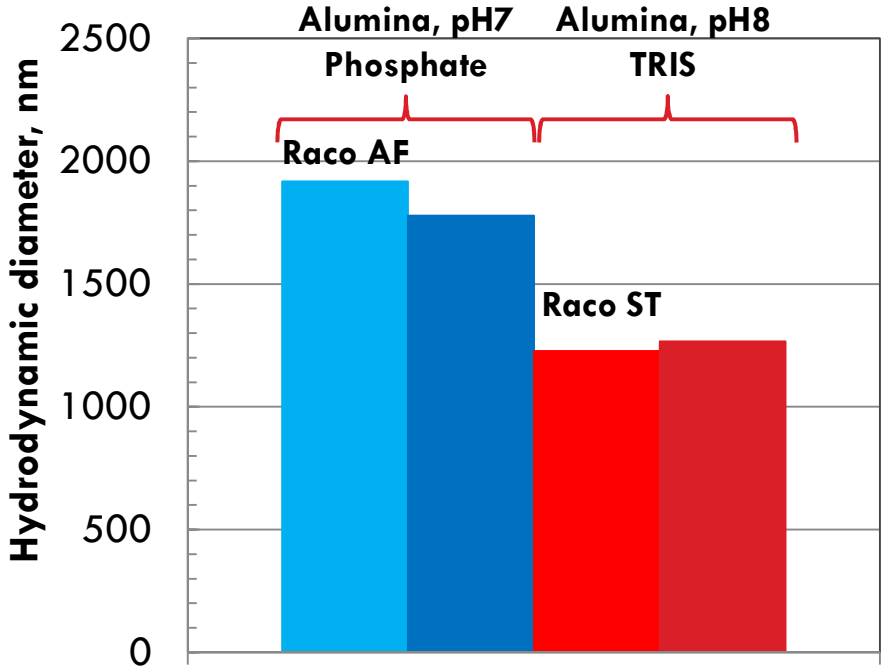




The presence of phosphate decreased the adsorption due to electrostatic repulsion and inhibit the anion ligand exchange of carboxyl groups of the mAb with the alumina.

**To use Alumina/TRIS at pH8 in the Racotumomab ST vaccine formulation**

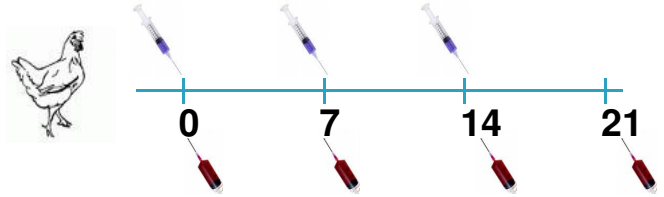
# New proposed formulation for Raco ST based on Alumina/ TRIS, pH8 showed lower median particles size than RacoAF/ Alumina/Phosphate buffer, pH 7 despite very low value of Zp

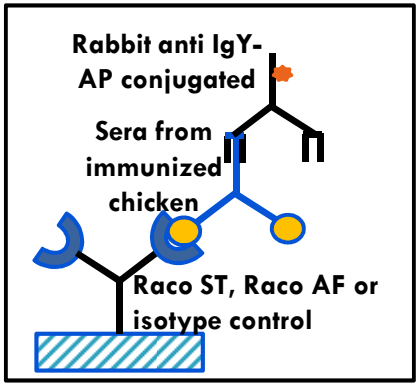



Electrostatic interaction plays a minor role on adsorption, but the ligand exchange reaction may be responsible of the stronger interaction between Racotumumab and alumina.

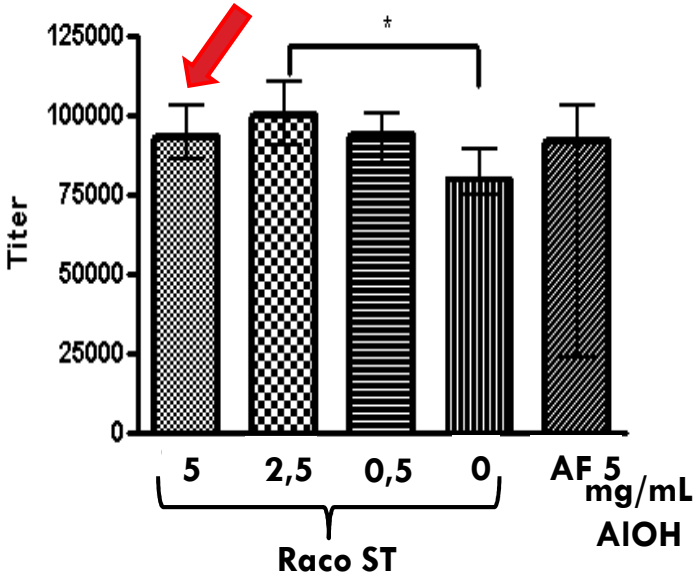
# Racotumomab/adjuvant ratio didn´t affect immunogenicity in chickens measured as the antibody titers against antigen, both IgG1 isotypic and idiotypic response



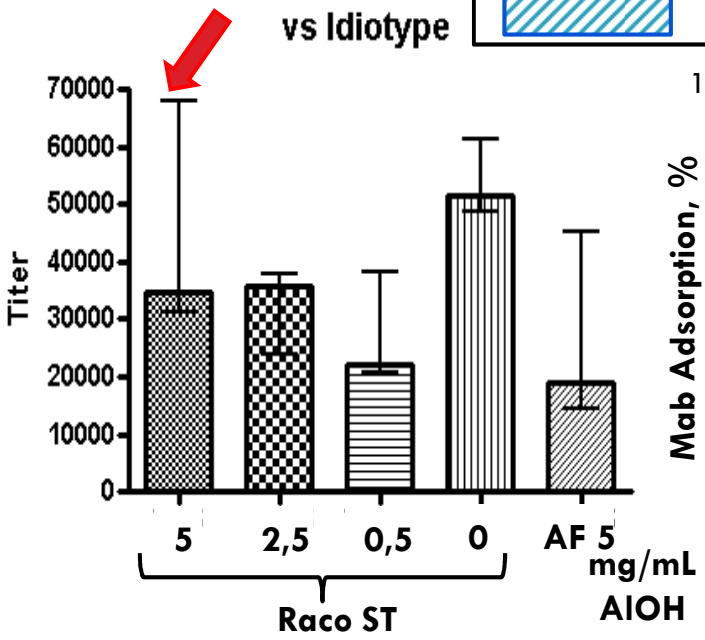



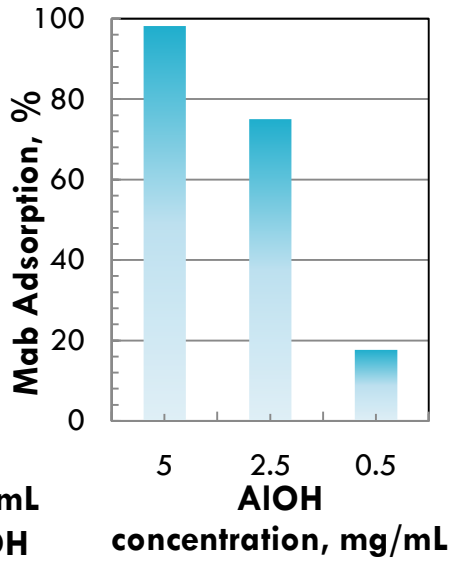

Test One-Way Anova

vs. Racotumomab



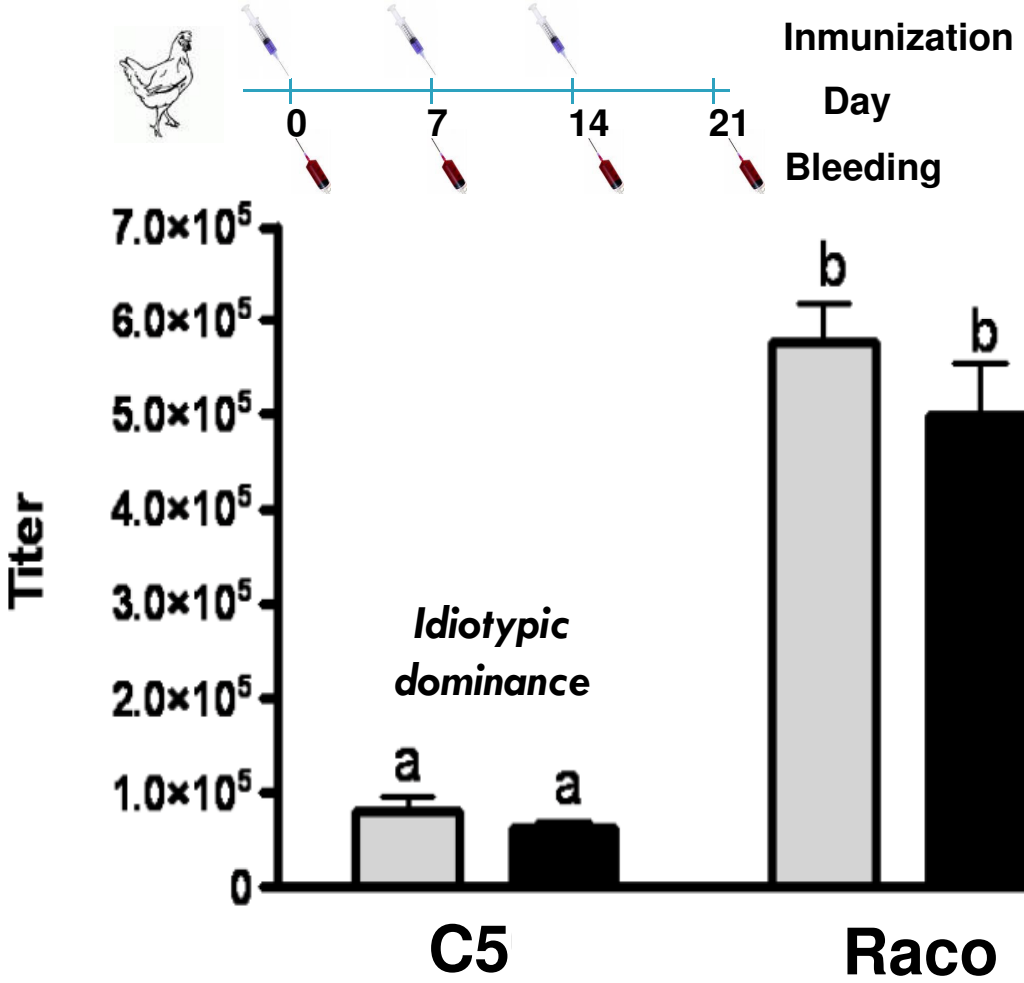

vs Idiotype



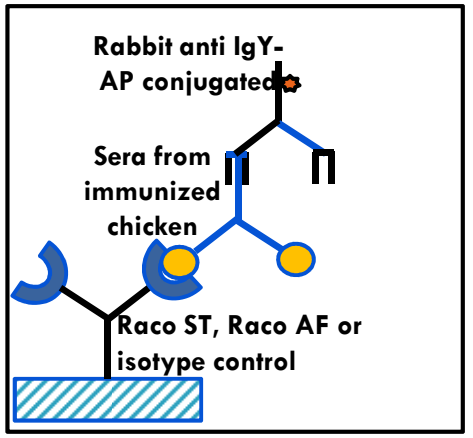

**To maintain 5 mg/mL of alumina in the vaccine formulation**

# Similar Ab3 antibody responses elicited by Raco-AF and ST vaccination in chicken



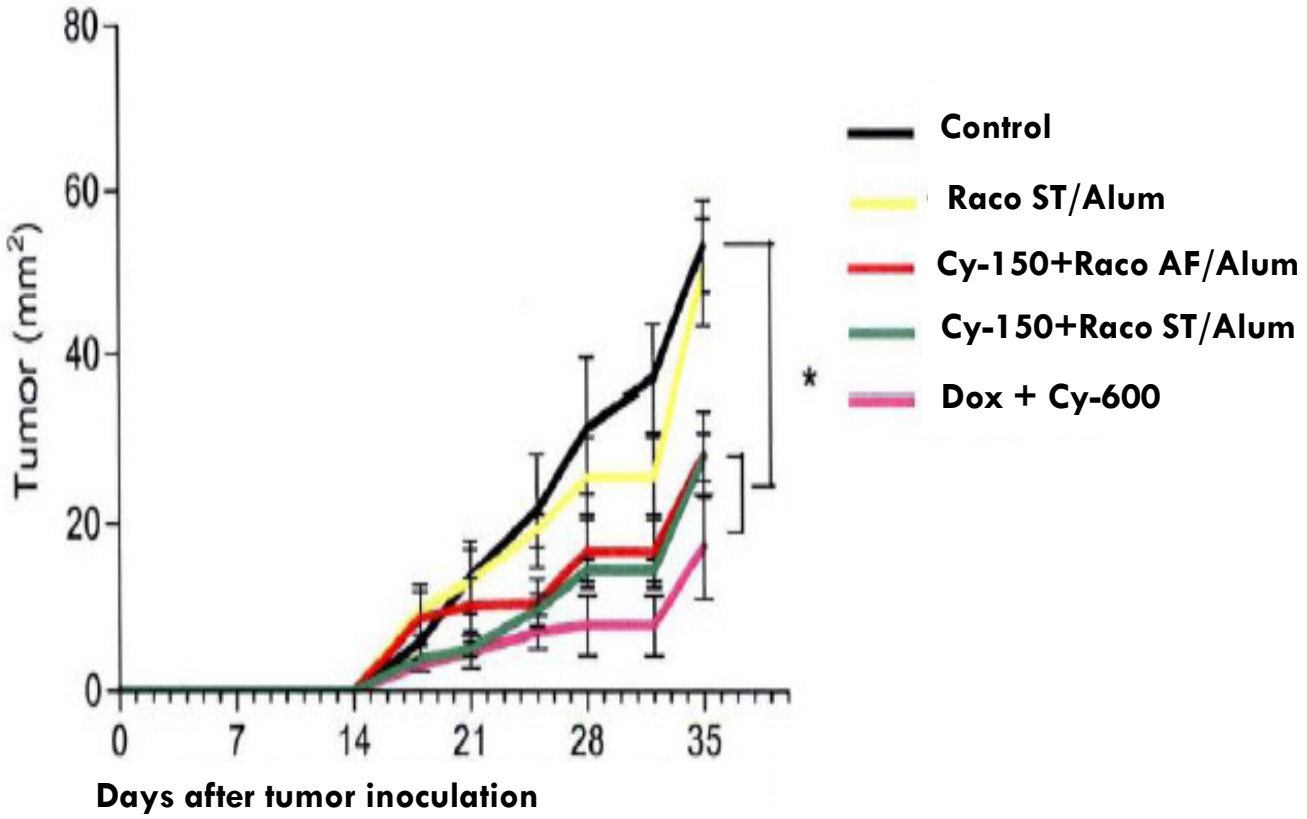

***Frequency of seroconversion to NGcGM3***

| | | |
|---|---|---|
| AF | 22/22 | 100 % |
| ST | 20/21 | 95% |




*Machado et al. BMC Biotechnology 2011, 11:112*

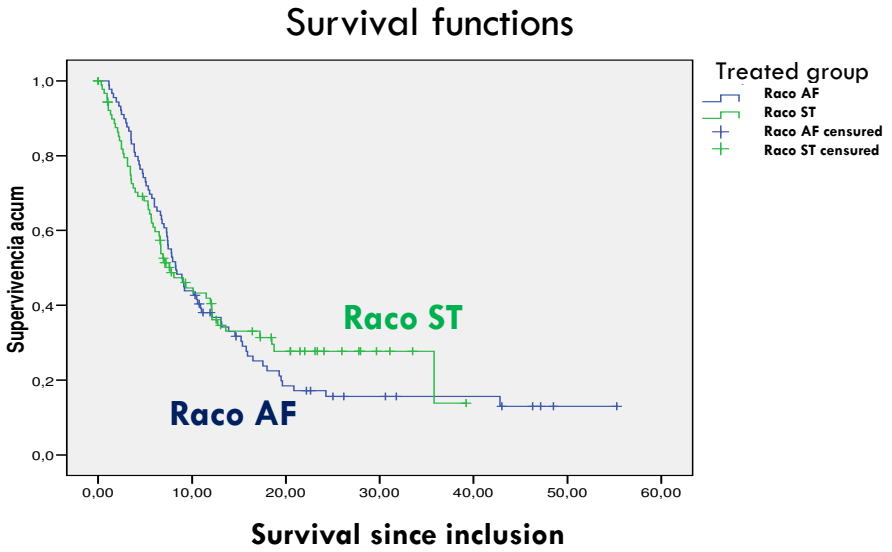
# Co-administration of Raco-AF/Alum or Raco ST/Alum with low-dose Cyclophosphamide induced anti-tumor effects in a mammary carcinoma model

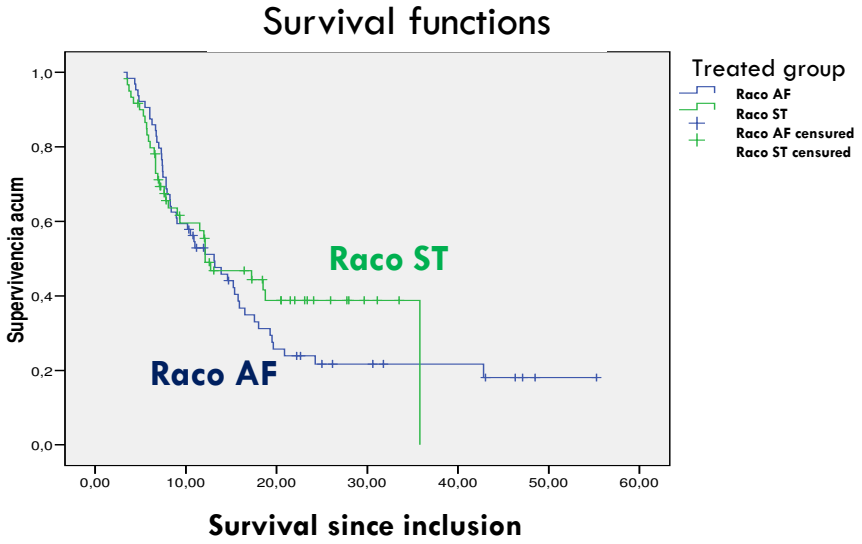



*Machado et al. BMC Biotechnology 2011, 11:112*

# Expanded Access Program (Population-based study) in patients with advanced stages (IIIB/IV) of NSCLC (recurrent and/ or metastatic)

**Intent to Treat. Per protocol: ≥ 5doses**




| OS (ITT) | | | | |
|---|---|---|---|---|
| Group | Mean months | Median months | SV rate 12m | SV rate 24 m |
| Raco ST N= 180, E= 132 | 16.6 | 8.06 | 39 | 21 |
| Raco AF N= 89 E= 73 | 15.6 | 8.26 | 38 | 17 |

| OS per protocol | | | | |
|---|---|---|---|---|
| Group | Mean months | Median months | SV Rate At 12m | SV Rate at 24m |
| Raco ST N= 124, E= 81 | 19.3 | 12.1 | 55 | 38 |
| Raco AF N= E= | 20.2 | 13.1 | 53 | 21.7 |

# CONCLUSIONS

- ✓ Here, we demonstrate that change of Racotumomab production from ascites to bioreactor generates a molecule with a high similarity of point of view of primary structure, but with some different physicochemical properties.
- ✓ Changes were observed in the degree of asparagine deamidation, C-terminal, lysine processing, methionine oxidation, and glycosylation pattern, that had an impact on the charge profile of the Mab molecule.
- ✓ Removal of phosphate and use of TRIS buffer at pH 8, maintaining 5 mg/mL of Alumina concentration in Racotumomab ST vaccine formulation allowed a higher and more stable adsorption level.
- ✓ No aggregates or increase in particle size were observed despite the low values of Z potential obtained with the new formulation.

# CONCLUSIONS

- ✓ However, observed changes did not affected the biological activity of the product, i.e. the idiotypic dominance (which represents the intended effect for an idiotypic vaccine) and the antitumoral effect in mice.
- ✓ Moreover clinical evaluation of vaccine based on Racotumomab produced by *in vitro* process confirmed results obtained using bio-models and were comparable with those obtained using *in vivo* based production process.
- ✓ Furthermore, our study demonstrated a number of physicochemical properties that do not affect the biological activity of the idiotypic vaccine, and by consequence should not be considered as critical attributes.
- ✓ Thus, the transfer of production process of idiotypic mAb from ascites to bioreactor improved product safety, without affecting its biological activity.

# Project team

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And more …..

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